

Listing of Claims:

The following is a marked-up version the Claims pursuant to revised 37 C.F.R. §1.121, with instructions and markings showing changes made herein to the Claims as filed.

Underlining denotes added text while strikeout denotes deleted text.

Claims 1-48 (Cancelled)

Please add the following new Claims:

49. (New) A method for producing a protein from an evolved microorganism comprising the steps of:

- a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein;
- b) culturing said microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type; and
- c) restoring said evolved microorganism to a wild type mutation rate.

50. (New) The method of Claim 49, wherein said evolved microorganism expresses said at least one heterologous protein.

51. (New) The method of Claim 50, further comprising the step of isolating said at least one heterologous protein from said evolved microorganism.

52. (New) The method of Claim 49, wherein said at least one heterologous protein is a hydrolase.

53. (New) The method of Claim 53, wherein said hydrolase is selected from the group consisting of proteases, esterases, lipases, phenol oxidase, permeases, amylases, pullulanases, cellulases, glucose isomerase, laccases, and protein disulfide isomerases.

54. (New) The method of Claim 49, wherein said microorganism comprises at least one copy of said mutator gene in its chromosome and said step of restoring said evolved microorganism to wild-type mutation rate comprises excision of said mutator gene.

55. (New) The method of Claim 49, wherein said mutator gene comprises at least one mutator gene selected from the group consisting of comprises *mutD*, *mutT*, *mutY*, *mutM*, *mutH*, *mutL*, *mutS*, and *mutU* mutations or homologues thereof.

56. (New) The method of Claim 55, wherein said mutator gene comprises at least one *mutD* mutation.

57. (New) The method of Claim 56, wherein said mutator gene comprises *mutD* mutations selected from the group of *mutD* mutations set forth in Table 1.

58. (New) The method of Claim 49, wherein said microorganism is selected from the group consisting of *E. coli* and *E. blattae*.

59. (New) The method of Claim 49, wherein said microorganism comprises a plasmid comprising the heterologous mutator gene and said step of restoring said evolved microorganism to a wild type mutation rate comprises curing the evolved microorganism of said plasmid.

60. (New) The method of Claim 59, wherein said plasmid comprises a temperature sensitive origin of replication.

61. (New) A method for producing a heterologous protein in an evolved microorganism comprising the steps of:

- a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein, wherein said at least one heterologous protein is an enzyme necessary for an enzymatic pathway;
- b) culturing said microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5 to 100,000-fold relative to wild type; and
- c) restoring said evolved microorganism to a wild type mutation rate.

62 (New) The method of Claim 61, wherein said enzyme is selected from the group consisting of reductases and dehydrogenases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of ascorbic acid or ascorbic acid intermediates.

63. (New) The method of Claim 62, wherein said enzyme is selected from the group consisting of glycerol dehydratase and 1,3-propanediol dehydrogenase, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of 1,3-propanediol, 1,3-propanediol precursors, and 1,3-propanediol derivatives.

64. (New) The method of Claim 62, wherein said enzyme is selected from the group consisting of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of glycerol and glycerol derivatives.

65. (New) The method of Claim 61, wherein said evolved microorganism expresses said at least one heterologous protein.

66. (New) The method of Claim 61, further comprising the step of isolating said at least one heterologous protein from said evolved microorganism.

67. (New) The method of Claim 61, wherein said microorganism is selected from the group consisting of *E. coli* and *E. blattae*.

68. (New) The method of Claim 61, wherein said microorganism comprises a plasmid comprising the heterologous mutator gene and said step of restoring said evolved microorganism to a wild type mutation rate comprises curing the evolved microorganism of said plasmid.

69. (New) The method of Claim 61, wherein said plasmid comprises a temperature sensitive origin of replication.

70. (New) The method of Claim 69, wherein said mutator gene comprises at least one *mutD* mutation.

71. (New) The method of Claim 61, wherein said mutator gene comprises at least one mutator gene selected from the group consisting of comprises *mutD*, *mutT*, *mutY*, *mutM*, *mutH*, *mutL*, *mutS*, and *mutU* mutations or homologues thereof.